THE LIFE HISTORY AND CYTOLOGY OF SACCOBLASTIA INTERMEDIA, N. SP.

DAVID H. LINDER

Mycologist to the Missouri Botanical Garden
Assistant Professor of Botany in the Henry Shaw School of Botany of Washington University

The genus *Saccoblastia*, of the Auriculariales, was first described from Brazil by Möller ('95) from material that he had collected and studied. Subsequently several representatives of the genus have been described from Europe, and one from North Carolina in the United States. While its distribution is of interest, the real importance of the genus is emphasized by the amount of speculation as to the life history of its members, especially from the cytological point of view. For this reason, the writer has thought it well worth while to present his observations on an as-yet-undescribed species that he collected in Cuba.

The species of *Saccoblastia*, so far as can be determined from the literature, are all effused and fall into two groups,—those that are floccose or hypochnoid, and those that are of a gelatinous or mucous consistency. To this latter group, Bourdot and Galzin ('27) have applied the subgeneric name of *Saccogloea*, and it is to this subgenus that the species under discussion belongs. Both macro- and microscopically, however, it is distinct from all species that have been described up to the present time. For purposes of comparison, certain measurements of this and the related species have been tabulated. From the data thus presented, it is

Species	Probasidium	Basidium	Spores
S. sebacea	6–9 × 18–30 µ	4–6–9 × 45–75 µ	5–8 × 8–16 µ
S. sebacea var. *pruinosa*	7–9 × 12–18 µ	4.5–6 × 45–60 µ	4.5–7 × 6–10 µ
S. subardosiaca	7–9 × 30–36 µ	———	6.8 × 15–18 µ
S. caroliniana	8.5–16 × 24–45 µ	———	7–7.7 × 15–17 µ
Saccoblastia sp.	7.2–11 × 28–39.6 µ	5.4–9 × 64–72 µ	5.4–8.1–9 × 14–19.8–24 µ

clear that the Cuban material has a close resemblance to that from North Carolina, although differing in the size of the spores. A character not shown in the table, and one of great interest, is the presence of a second type of probasidium which is clavate and resembles that of the genus *Iola.* This additional character, together with the fact that the colonies are not effuse but pustulate, makes it desirable to recognize this as a new species to which the name *Saccoblastia intermedia* is given because of the apparently transitional position between *Iola* and *Saccoblastia.*

Saccoblastia intermedia n. sp.

Fructifications pustulate, white to dilute brown, gelatinous to mucous, up to 1 cm. diam., becoming somewhat pendant at maturity. Context hyaline, the hyphae slender, 2.7–5.4 μ thick, without clamp connections. Probasidium of two types, the clavate type hyaline, rarely slightly constricted in the middle, 5.4–9 × (18)–36–45 μ, the walls slightly thickened; the lateral probasidium saccate, pendant, hyaline, 7.2–11 × 28–39.6 μ. Basidia hyaline, straight or slightly arcuate, 5.4–9 × 64.8–72 μ, 3–(5)-septate, bearing 4, less frequently as many as 6, sterigmata of varying length, 1.8–3.6 × 7.2–50 μ, the shorter ones conical. Basidiospores 1– rarely 2-celled, ellipsoid to elongate-ellipsoid, frequently concave on one side, tapering abruptly and obliquely to short truncate apiculi.

On moist decaying stump, Soledad, Cuba, September, 1924, *Linder* (TYPE in Farlow Herbarium, Herbarium of the Missouri Botanical Garden, and in the writer's herbarium).

Life History and Cytology

The material upon which the following account is based was preserved in alcohol of approximately 70 per cent strength. For study it was mounted in lactophenol to which had been added cotton blue and picric-nigrosin. The specimens as thus preserved in alcohol were plasmolyzed and the finer details of nuclear structure were lost. The lactophenol, however, practically restored the cytoplasmic structure, and as a result the nuclei in most of the material became clearly visible and were stained by the combination of cotton blue and picric-nigrosin.[1]

Examination of the pustules under the microscope reveals the fact that the fruiting body is not highly developed. The hyaline

[1] Weston, W. H. A useful modification of Amann's medium. Science N. S. **70**: 455. 1929.

hyphae growing out radially in the gelatinous matrix soon give rise to peculiarly twisted or irregularly waved hyphae that connect two or more of the main hyphae (pl. 39, fig. 2, and pl. 41, fig. 1). The walls of these connectives are somewhat thicker than are those of the remainder of the vegetative hyphae, and are also slightly, though distinctly, colored brown. The peculiar growth indicates that the junction of the two hyphae is more than a mere anastomosis and that the wavy development is in reality the result of a stimulus that is sexual in nature. In other words, the connectives appear to take the place of clamp connections. Additional credence is given to this supposition by the abundance of such hyphae in the region below the first-formed probasidia. This is clearly shown in pl. 39, fig. 2, where the clavate probasidia are formed immediately above the junction of the hyphae with the connective.

The clavate probasidia are produced terminally on hyphae or on the ends of branches from them, by swelling. At the initiation of probasidium formation the protoplasm in the region involved becomes dense, and the nuclei are clearly seen in pairs for the first time. As enlargement of the tip of the hypha proceeds, the paired nuclei increase slightly in size and then migrate into the now clavate body (pl. 41, figs. 2–4) where they shortly fuse. The resulting nucleus is larger and, with its more deeply staining nucleolus, is clearly visible.

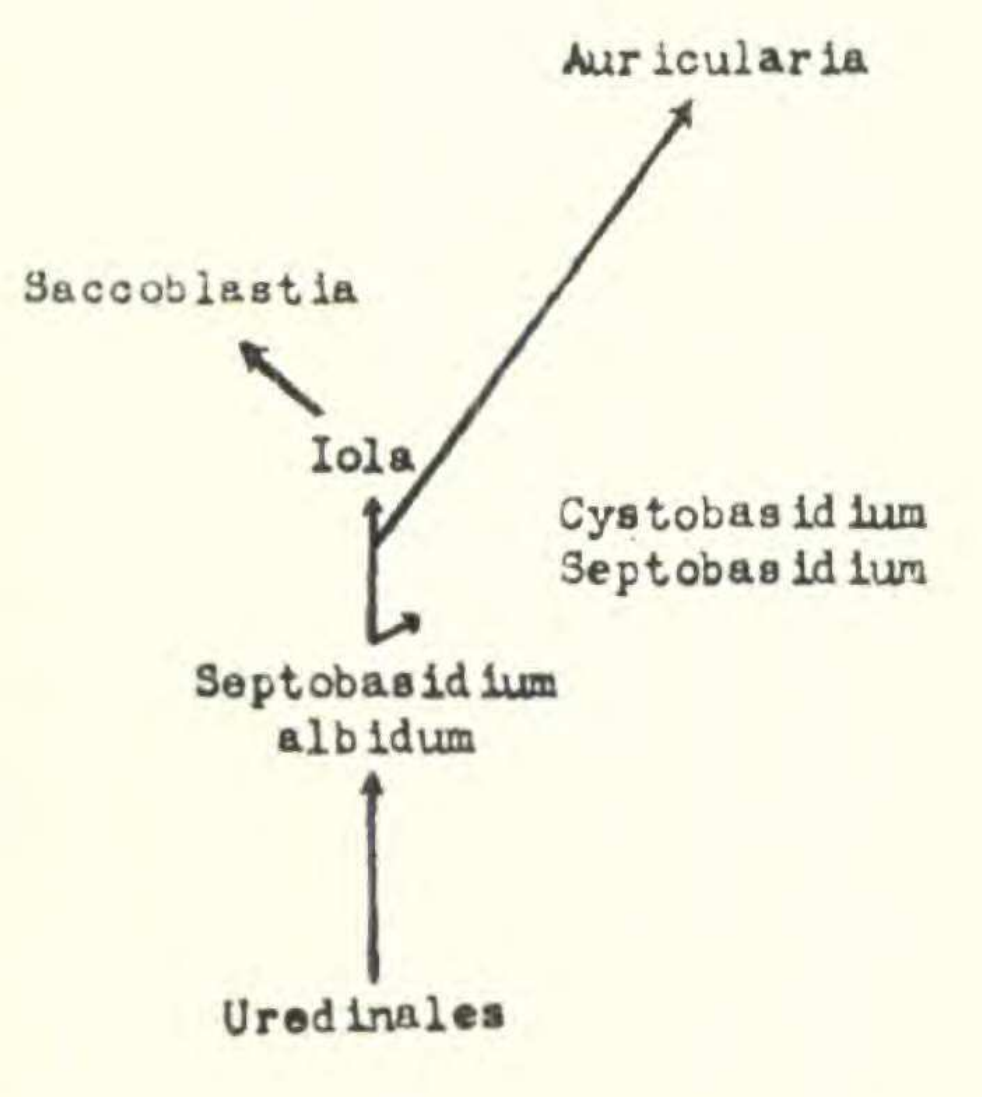
Just as in the clavate type of probasidium, the saccate type is also the seat of nuclear fusion, the only difference being that the paired nuclei migrate laterally into the sac-shaped outgrowth from the main hypha as is shown in pl. 40, figs. 1–3, and pl. 41, figs. 5–7. The saccate probasidia, although produced concurrently with the clavate type, do not appear until later in the life history of the fungus. If, as has been assumed earlier in this paper, *Saccoblastia intermedia* is transitional between *Iola* and *Saccoblastia*, then it seems possible to assume the saccate probasidia to be a secondary type that allows proliferation, and is the type that has become fixed in the remaining species of *Saccoblastia*. Not infrequently, the growing point of the saccate probasidium, the base in the earlier stages of development, continues its growth by producing a germ-tube (pl. 40, fig. 4, and pl. 41, fig. 9) in which case it appears to be transitional between the two types of probasidia. Also, although more rarely, the saccate type may germinate at the basal and apical end (pl. 41, fig. 10). Usually, however, such exceptions are infrequent, and the normal method of germination is by apical growth (pl. 41, fig. 8). At all events, the paired nuclei migrate laterally into the small immature probasidium and very quickly fuse. The rapidity with which this migration and fusion take place becomes evident when the sizes of the sacs, shown in pl. 41, figs. 5–7, are compared.

After a short rest, the probasidium sends forth a long hypha, or promycelium, which eventually enlarges terminally to become the true basidium. The fusion nucleus remains behind in the probasidium until the hypha attains full length, then there is a rapid migration of the nucleus into the basidium, followed by the entire protoplasmic contents of the probasidium and hypha (pl. 41, fig. 1). That the migration of the nucleus is rapid can only be inferred from the fact that the nucleus has never been observed by the writer anywhere but in the probasidium or the true basidium, never in the hypha connecting the two. Once the basidium is formed, it is most frequently 3-septate and each of the resulting cells has but a single nucleus. The nucleus and the protoplasmic content of each cell eventually migrate into the spore as it is formed so that the probasidium after it has produced the spores is nothing but an empty shell (pl. 39, fig. 1, and pl. 41, figs. 13 and 17).

Discussion

From the above account of the life history of this species it is obvious that, with the fusion of the paired nuclei taking place in the probasidium, that organ is actually homologous with the teleutospore of the rusts. Furthermore, the manner of germination of the basidium, that is, spore production, is strikingly like that found in the rusts. The resemblance between the probasidium and the teleutospore has been remarked upon since the genus *Saccoblastia* was first described. Möller ('95) in his account of the genus says: "Auch unter den Uredinaceen giebt es ja Formen, bei denen die Teleutospore ohne längere Ruhepause unmittelbar zur Basidie auskeimt. Durch diese Formen wird die nahe Verwandtschaft unserer Saccoblastia-Arten mit den Uredinaceen besonders deutlich." Coker ('20) also mentions this relationship in the following extract from his account: "This genus reminds one strongly of the rusts, particularly the genus Gymnosporangium, where the teleutospore sprouts as soon as formed. If the relationship is as close as it seems the pear-shaped sac would be the homologue of the teleutospore. Note that this sac when long is constricted in the middle." While other writers point out this affinity, Coker is the only one who implies that the genus is derived from the rusts. Thus, Möller ('95), Neuhoff ('24), and Gäumann ('26), in their phylogenetic trees, indicate that this genus and the family to which it belongs, the Auriculariaceae, arose before the rusts. The main objection to Möller's scheme of evolution is based on the fact that in the Ustilaginaceae the nuclei in the cells of the basidium divide into two daughter nuclei, one of which migrates into the spore, the other remaining in the basidial cell. In *Saccoblastia* such is not the case, for the nucleus migrates into the spores with the protoplasm of the cell after the manner of *Gymnosporangium* or other members of the Pucciniaceae. To Neuhoff's and Gäumann's system it may be objected that a form with a relatively simple life-cycle should not be selected as the source of forms with a very complicated one.

From the nature of the subject, discussions of phylogeny are highly theoretical, yet it seems to the writer that the evidence pointing to the derivation of the Auriculariales from the Uredinales is stronger than the reverse. The evolutionary tendency seems to be from the rusts to *Auricularia* somewhat in the manner shown briefly in the accompanying text-figure. It is quite possible that the group as a whole is polyphyletic in its origin, but in this scheme the chief idea is to show trends in certain characteristics. Thus we find among the Pucciniaceae a tendency to drop out one or more steps in the life-cycle. Also, as is true in *Gymnosporangium*, there is a marked shortening of the



rest period of the teleutospore. If now it can be supposed that during the past history of the family, either through gradual evolution or through mutation, a race arose that was weakly parasitic or even saprophytic, then we arrive at the forerunner of a genus similar to *Septobasidium* or *Cystobasidium* in which the thickened walls of the probasidium still are in evidence. In this connection, it is unfortunate that Patouillard ('13) was unable to make a cytological study of the toruloid spores of *Septobasidium albidum*. Had careful investigation shown the spores to be binucleate, then there would have been demonstrated an even closer relation between the rusts and *Septobasidium* since in that case the toruloid spores might with reason be considered homologous with aecidiospores.

The next step in advance would be a genus similar to *Iola* of which the probasidium has practically lost its thickened walls. Through *Saccoblastia intermedia* with its clavate and saccate probasidia there appears to be a natural transition from *Iola* to *Saccoblastia*. The fact that the clavate probasidia are the first produced, and that they are found only in the older material, is significant. Since the clavate type is terminal, it is apparent that in order to produce additional probasidia with the least waste of material the subsequent probasidia must be produced laterally from the binucleate hyphae. This is what appears to have happened, but the remaining members of the genus *Saccoblastia* have retained the saccate type to the exclusion of the clavate.

Reduction of the probasidium is continued until in *Auricularia* we find, according to Gäumann and Dodge ('28, page 542), the place of fusion of the nuclei indicated only by a slight swelling. The branch of the family tree terminated by *Auricularia* appears to have arisen slightly before *Iola* since the latter genus apparently has lost the asexual method of reproduction. In *A. auricula-judae* the paraphyses have been shown by Sappin-Trouffy ('96) to be binucleate. These binucleate cells might be considered as homologous with the conidia produced by *Septobasidium albidum*, although they have lost their reproductive function. The binucleate condition of the spores of *S. albidum* would not be unparalleled, for Dangeard ('95) has shown the presence of binucleate oidia in *Dacryomyces deliquescens* of the Dacryomycetales. Should the conidia of *S. albidum* or other species of *Septobasidium* eventually prove to be binucleate, then there would be slight reason for doubting the homology between such conidia and aecidiospores.

Throughout this theoretical discussion, it will be observed that the present hypothesis is founded on the reduction of the teleutospore or probasidium, on the reduction of the aeciospores, on the development of an extensive fruiting body, and finally on the transition from the parasitic mode of existence to the saprophytic. Still another phase of the subject that has been considered, though not discussed for lack of available knowledge, is the relation of the sporidia to pycnidiospores. Brefeld ('88), however, has remarked on the resemblance between the two, and has called attention to the fact that he was unable to obtain germination of the sporidia.

It is not necessary to consider the sporidia homologous with the pycnidiospores, although a study of the cytology and the relationship of these spore forms would undoubtedly bring to light much very interesting information. To the writer's way of thinking, it is sufficient to realize that the morphology of the basidiospores of the rusts and Auriculariales is fairly constant throughout the groups. Therefore, to find that the spores of certain rusts, such as *Cronartium ribicola*, also produce sporidia is sufficient evidence to show that there is a very close relationship between the two orders.

All factors considered, and in the light of present-day knowledge, it would appear that the Auriculariaceae are derived from the Uredinales through the reduction or degeneration of the reproductive organs as a result of the saprophytic mode of existence. To have the Uredinales derived from the Auriculariales would presuppose the development of potentialities in organs or even the development of the organs themselves. The probasidia of the Auriculariales have all the evidence of being vestigial structures handed down from the Uredinales, even though the latter order be derived from the Red Algae, the Zygomycetes, or even, as seems most probable, from a parasitic group of Ascomycetes.

Bibliography

Bourdot, H. et A. Galzin ('27). Hymenomycetes de France **1**: 4–5. 1927.

Brefeld, O. ('88). Unters. a. d. Gesammtgeb. d. Myk. **7**: 1–178. *pl. 1–11.* 1888.

Coker, W. C. ('20). Elisha Mitch. Scientif. Soc. Jour. **35**: p. 121. *pl. 33, 53.* 1920.

Dangeard, P. A. ('95). Le Botaniste **4**: 119–181. *figs. 6–7.* 1895.

Gäumann, E. ('26). Vergleich. Morph. Pilze. 626 pp. Jena, 1926.

————, and C. W. Dodge ('28). Comp. Morph. Fungi, 701 pp. New York, 1928.

Möller, A. ('95), in Schimper, Bot. Mitt. a. d. Tropen **8**: 1–179. *pl. 1–6.* 1895.

Neuhoff, W. ('24). Bot. Archiv **8**: 250–297. *pl. 1–4, 7 text-figs.* 1924.

Patouillard, N. ('13). Compt. Rend. Acad. Sci. Paris **156**: 1699–1701. *figs. 1–2.* 1913.

Sappin-Trouffy, P. ('96). Le Botaniste **5**: 53–58. *figs. 3–6.* 1896.

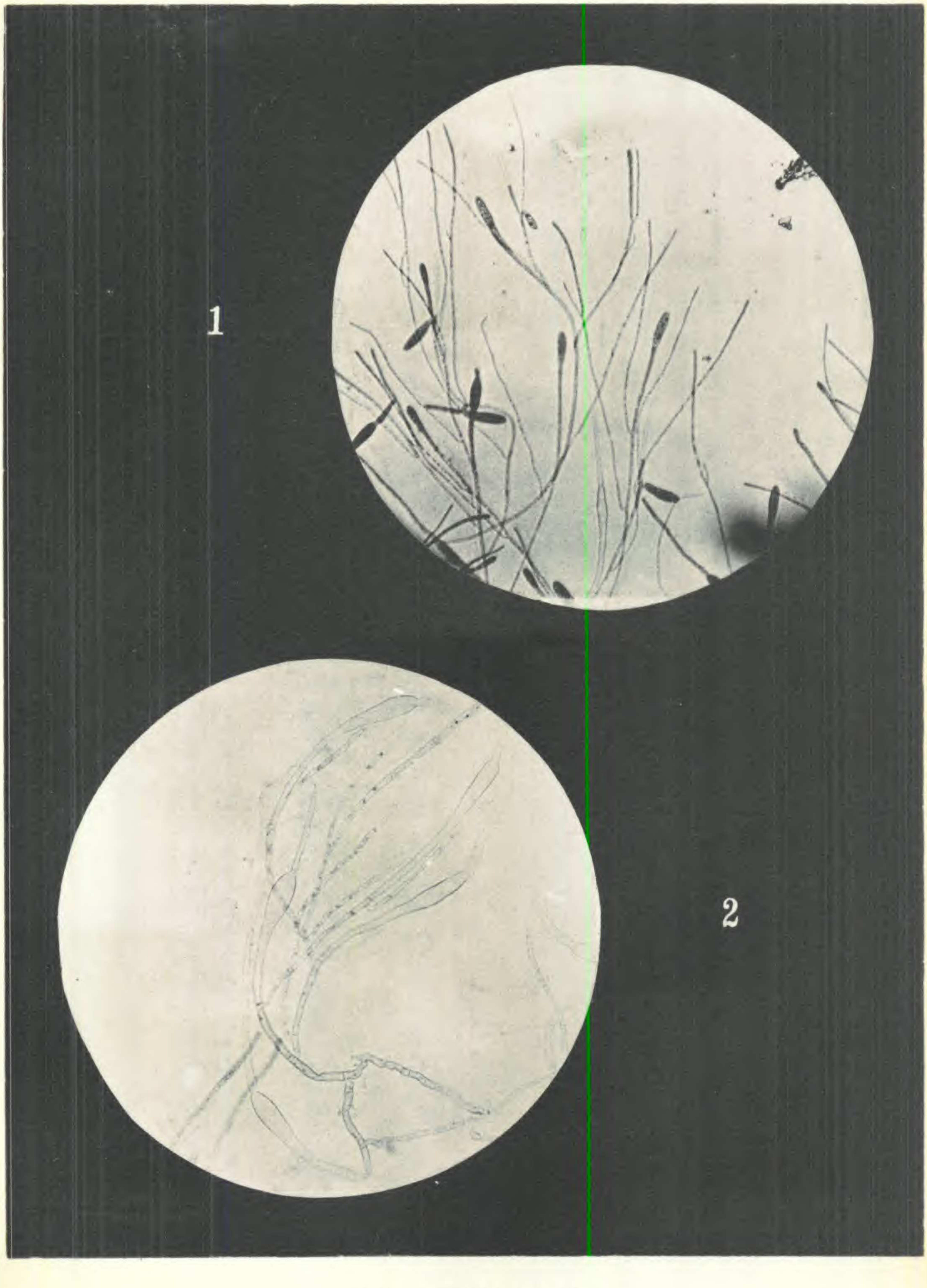
Explanation of Plate

PLATE 39

The material from which all photographs and drawings were made was mounted in Amann's lactophenol to which had been added cotton blue and picric-nigrosin. The photographs were made on Eastman panchromatic plates with the aid of a Wratten "A" (red) filter.

Fig. 1. Photograph at a low magnification to show the characteristic features of *Saccoblastia intermedia.* In the middle of the picture, near the bottom, will be seen an empty probasidium which has given rise to a true basidium, barely outlined above. The latter has been emptied of its contents through the production of basidiospores. An immature saccate probasidium with its fusion nucleus is shown to the left of the empty basidium, while immediately below the point of attachment of the probasidium, on the same hypha, are paired nuclei that have not fused. Below these are two nuclei that are approaching the paired condition. Approx. × 120.

Fig. 2. Clavate probasidia are shown arising after the fusion of the connective hypha with the main one. Paired nuclei may be observed in the hyphae between the two uppermost probasidia. Approx. × 240.



LINDER—SACCOBLASTIA

COCKAYNE, BOSTON

Explanation of Plate

PLATE 40

The magnification in all instances is approximately × 700.

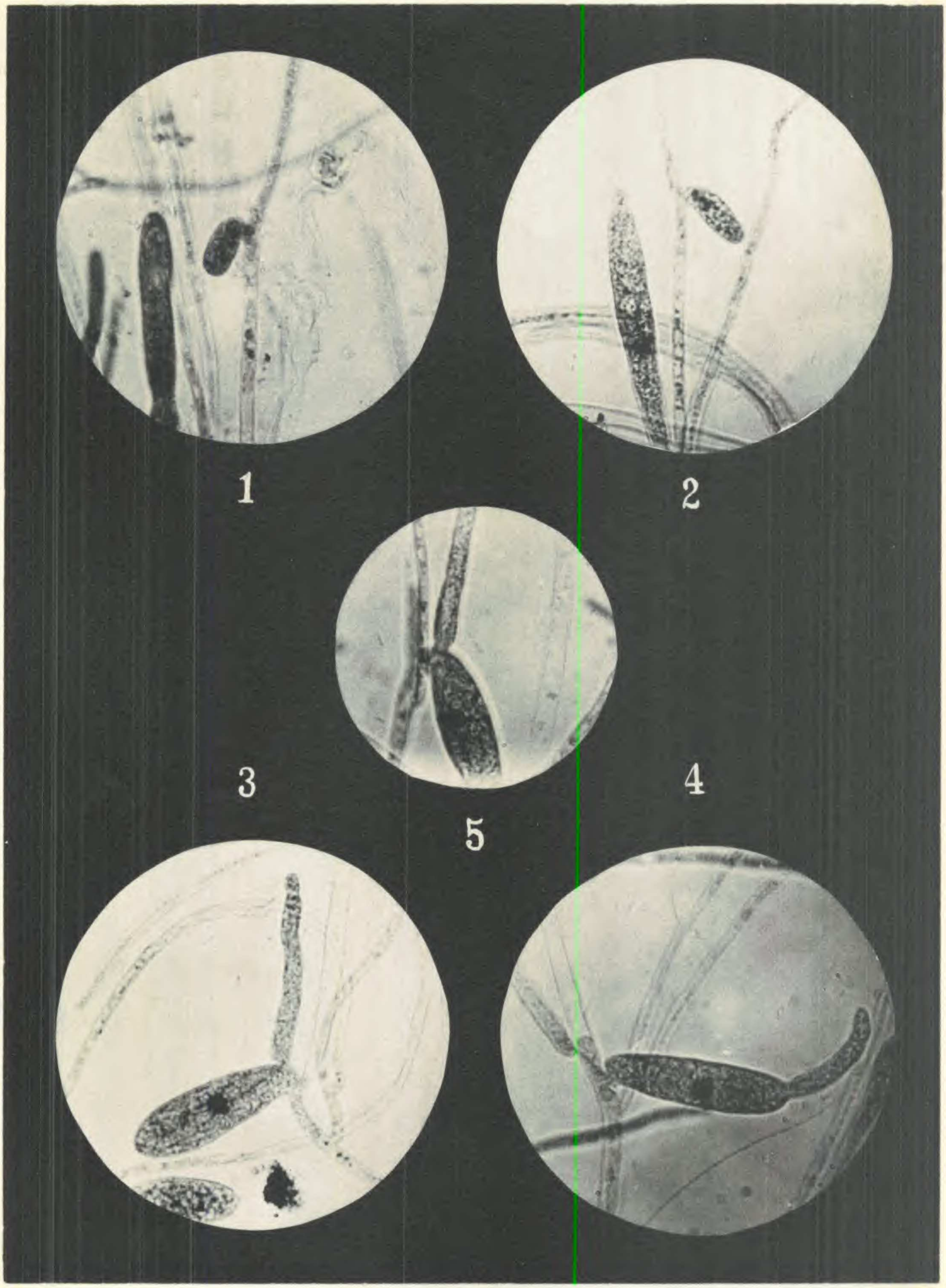
Fig. 1. A young probasidium into which one of the paired nuclei has migrated. The other nucleus is about to enter. Below and on the same hypha are two nuclei which have associated in pairs. See also pl. 41, fig. 5.

Fig. 2. A young probasidium in which the two nuclei have become closely associated and are about to fuse. See also pl. 41, fig. 6.

Fig. 3. A germinating probasidium with its conspicuous fusion nucleus.

Fig. 4. A probasidium continuing apical growth by the production of a promycelium from what is ordinarily the basal end.

Fig. 5. A germinating probasidium showing the conspicuous nucleus with its more deeply staining nucleolus. The promycelium is more than four times as long as this particular probasidium.



LINDER—SACCOBLASTIA

COCKAYNE, BOSTON

Explanation of Plate

PLATE 41

All drawings have been made with the aid of a camera lucida and are shown at a magnification of × 600.

Fig. 1. Shows the peculiarly wavy and somewhat twisted connective hypha that is formed prior to the production of probasidia. Such hyphae appear to take the place of clamp connections. See pl. 39, fig. 2.

Figs. 2–4. Formation of the clavate type of probasidium.

Figs. 5–8. Formation of the saccate type of probasidium. Figs. 5 and 6 are drawn from the same probasidia as are shown in pl. 40, figs. 1–2.

Figs. 9–10. The abnormal germination of probasidia. In fig. 9 the promycelium is arising from the basal end of the probasidium, while in fig. 10 each end is giving rise to a promycelium.

Figs. 11, 13–15. Types of true basidia. In fig. 13 spores have been produced but only one remains attached. It will be noted that not all of the protoplasm of the basidial cell has been able to enter the spore. In the remaining basidia are shown variations in the size of the sterigmata and number of septations of the basidia.

Fig. 12. A germinating basidiospore.

Fig. 16. Basidiospores, all of which contain but one nucleus apiece.

Fig. 17. A probasidium which has given rise to a promycelium and basidium. The promycelium is somewhat shortened After the basidium has produced the basidiospores, it collapses and finally disintegrates. Compare with that shown in pl. 39, fig. 1.